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**WO 01/043540 A3**

(54) Title: METHODS FOR PRODUCING TRANSGENIC ANIMALS

(57) Abstract: The present invention relates to methods for producing transgenic animals. Specifically, the methods of the present invention include production of a transgenic animal by transgenic intracytoplasmic sperm injection, retroviral gene transfer, intracytoplasmic nuclear injection, and pronuclear injection. In addition, the present invention also relates to methods for using transgenic animals as models for human disease and diagnosis. In particular, these transgenic animals may be used as models for embryo and fetal development, as models to assess the safety and efficacy of drug therapy and gene therapy, and as models for disease diagnosis. The methods of the present invention are also directed to methods of using transgenic embryonic cells to treat human diseases.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/34011

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC 7 A01K67/027 A61K49/00 C12N5/06   |   |  |
|---|---|--|
| According to International Patent Classification (IPC) or to both national classification and IPC   |   |  |
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC 7 A01K A61K C12N  |   |  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched   |   |  |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)<br>EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, CHEM ABS Data, EMBASE   |   |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |   |  |
| Category *  | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
| X   | PERRY A ET AL: "Mammalian transgenesis by intracytoplasmic sperm injection" SCIENCE., vol. 284, 14 May 1999 (1999-05-14), pages 1180-1183, XP000919050 cited in the application   | 1,2, 5-19, 22-32, 36-42  |
| Y   | figure 2; table 2   | 3,4,44, 47-64  |
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| -/--  |   |  |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.   |   |  |
| * Special categories of cited documents :<br>"A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier document but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed<br>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br>"8" document member of the same patent family |   |  |
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| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.<br>Fax: (+31-70) 340-3016  |   | Authorized officer<br><br>Lonnoy, O                                    |

## INTERNATIONAL SEARCH REPORT

International Application No

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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|--|---|-----------------------|
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| Y  | WO 98 41615 A (WISCONSIN ALUMNI RES FOUND)<br>24 September 1998 (1998-09-24)<br>cited in the application<br>examples 6,7<br>---   | 44-64                 |
| P,X  | WO 00 09674 A (UNIV HAWAII)<br>24 February 2000 (2000-02-24)<br>the whole document<br>---   | 1-43,79               |
| P,X  | WO 00 08924 A (PERRY ANTHONY C F ;WAKAYAMA<br>TERUHIKO (US))<br>24 February 2000 (2000-02-24)<br>the whole document<br>---  | 1-43,79               |
| P,X  | CHAN A ET AL: "Foreign DNA transmission<br>by ICSI: injection of spermatozoa bound<br>with exogenous DNA results in embryonic<br>GFP expression and live rhesus monkey<br>births"<br>MOL HUM REPROD,<br>vol. 6, no. 1, January 2000 (2000-01),<br>pages 26-33, XP001002490<br>cited in the application<br>the whole document<br>--- | 1-43,79               |
| A  | WO 99 25863 A (HOVATTA OUTI ;WINSTON<br>ROBERT (GB); IMPERIAL COLLEGE (GB); CEDARS<br>S) 27 May 1999 (1999-05-27)<br>page 9, line 2; claims 19,31<br>---  | 1,44,75               |
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| A  | SIMONI M: " Transgenic animals in male<br>reproduction research"<br>EXPERIMENTAL AND CLINICAL ENDOCRINOLOGY,<br>vol. 102, no. 6, pages 419-433,<br>XP001002682<br>---   |                       |
| T  | CHAN A ET AL: "Transgenic monkeys<br>produced by retroviral gene transfer into<br>mature oocytes"<br>SCIENCE.,<br>vol. 291, no. 5502,<br>12 January 2001 (2001-01-12), pages<br>309-312, XP002168955<br>-----   |                       |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/34011

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see further information sheet invention 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-64, 75,  
79 (all totally) and 76-78 (all partially)

- 1.1. Claims: 1-43,  
79 (all totally) and 76-78 (all partially)

Method for producing a transgenic animal comprising the step of transferring exogenous DNA by intracytoplasmic sperm injection into an oocyte, wherein said exogenous DNA is bound to the spermatozoon.

- 1.2. Claims: 44-64 (all totally) and 76-78 (all partially)

Method for producing a transgenic animal comprising the step of transferring exogenous DNA by intracytoplasmic sperm injection into an oocyte, wherein said exogenous DNA is provided as a retroviral particle.

- 1.3. Claims: 75 (totally) and 76-78 (all partially)  
Method for producing a transgenic animal comprising the step of transferring exogenous DNA by intracytoplasmic sperm injection into an oocyte, wherein said exogenous DNA is provided by pronuclear injection.

2. Claims: 65-69 (all totally) and 76-78 (all partially)

Method for producing a transgenic primate comprising the step of injecting a nucleus into an enucleated oocyte by intracytoplasmic nuclear injection, wherein said nucleus is isolated from a blastomere

3. Claims: 70-74 (all totally) and 76-78 (all partially)

Method for producing a transgenic primate comprising the step of injecting a nucleus into an enucleated oocyte by intracytoplasmic nuclear injection, wherein said nucleus is isolated from a somatic cell.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/34011

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)  | Publication<br>date  |
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